THE INTERNAL ANATOMY OF TWO PHALLOSTETHID FISHES

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INTRODUCTION

The Phallostethidae are highly specialized little fishes found in fresh and brackish water in certain limited regions of the Malay peninsula and the Philippines. They are of general interest in that they appear to provide a unique example of change of structure and function in a typical teleost organ, the pelvic fins of the male. The females are also modified, but to a lesser degree. The fishes derive their name from a large, muscular copulatory organ, the "priapium," suspended below the head in the male and which contains, in addition to the ductus deferens, the terminal apertures of the digestive and excretory systems (Figs. 1 and 2). In the female, the organ outlets are also anterior but, since there is no copulatory organ, the apertures are located on the ventral surface of the gular region (Fig. 3).

The phallostethids were first described and named by Regan in 1913 from a single species, but he notes a clear reference to their type by Duncker in 1904. Since Regan's work, twelve species have been discovered (Myers, 1928, 1937; Aurich, 1937). All are small, measuring from 14 to 40 mm. in length, and in 1935, after considerable study, Myers created for the group a new sub-order, Phallostethoidea, in the order Percesoces. Myers lists seven genera; Aurich groups them into three families and adds an eighth genus, *Solenophallus*.

Investigations on this group have been largely concerned with attempts to discover its taxonomic relationships, and a search has been made for possible homologies between the bony elements supporting the priapium and the bones of more typical teleosts.

Regan (1916), Bailey (1936) and Aurich (1937) have described the osteology of the priapium in several species of phallostethids. Bailey and Aurich each conclude that the priapial bones represent greatly modified pelvic fin and girdle elements, together with the first pair of ribs and, Bailey adds, possibly certain pterygials or rays of a portion of the pectoral fins. Aurich strengthens the argument that pelvic fin elements

have been transformed into priapial structures by describing the presence of vestigial fin rays in two species of the genus *Solenophallus,* and in *Gulaphallus mirabilis.* In all three species, he finds two or three delicate rays lying free in the skin behind the anus, and a second group of three rays inserted on the posterior extremity of the axial bone, a skeletal support which extends throughout the length of the priapium (see Aurich Fig. 3). In *Solenophallus,* these latter rays become comb-like at the ends. The females of several species possess a pair of papillae on either side of the oviduct opening (Regan, 1913; Herre, 1925, 1926; Villadolid and Manacop, 1934; Bailey, 1936). These may be vestiges of pelvic fins, and it is thought (Bailey) that the minute bony supports found in them in *Phenacostethus* may be remnants of the pelvic girdle. Villadolid and Manacop (1934) describe the development of the copulatory organ in *Gulaphallus,* showing it to arise from paired lobes surrounding the anus, on the ventral side of the throat. One of these lobes soon outgrows the other; the two fuse and gradually produce the elongate, asymmetrical priapium.

Brief descriptions of the soft parts of phallostethids have been published by Regan (1916) for *Phallostethus dunckeri* and *Neostethus lankesteri,* and by Villadolid and Manacop (1934) and Bailey (1936) for *Gulaphallus mirabilis.* There has been no detailed study of the gross and histological structure of the viscera, however, and it is to Dr. Hugh M. Smith that I owe the suggestion that such a study in these greatly modified forms would be of interest. Dr. Smith referred me to Dr. George S. Myers who supplied preserved material of two species, *Phenacostethus smithi,* and *Gulaphallus mirabilis,* from the collection at the U. S. National Museum. I wish also to acknowledge with appreciation the help Dr. Myers has given me in sending me a number of papers on the phallostethids.

Material and Methods

Phenacostethus smithi, described by Myers in 1928 from material collected by H. M. Smith in a fresh water stream in Siam, is the smallest phallostethid known (14–17 mm. total length) and is next to the minute Philippine goby, *Mistichthys luzonensis* (11–16 mm. total) in size. *Gulaphallus mirabilis* was described in 1925 by Herre as one of two species of a new genus which he had found in the fresh water streams of Luzon, the Philippine Islands. Its total length is not over 35 mm.

The specimens at my disposal were fixed in formalin and alcohol and preserved in alcohol. Transparent preparations of the skeleton were made by staining with alizarin and clearing in KOH and glycerine.

These were studied and dissected under a binocular dissecting microscope and other unstained specimens were dissected in alcohol for a study of organ arrangement. Sketches were made with the aid of a camera lucida. Serial sections were cut in transverse, sagittal, and frontal planes, 7–12 μ thick, and stained in iron haematoxylin, alum haematoxylin and triosin, and in Mallory's triple connective tissue stain. In all cases, fixation was poor for an adequate histological study and much more satisfactory results could be obtained with properly fixed material.

Skeleton

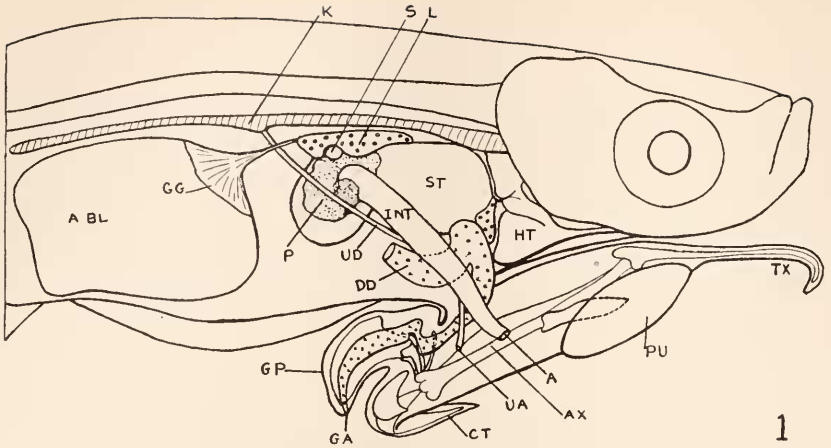
The descriptions of almost the entire skeleton of *P. smithi* and of *G. mirabilis* by Bailey (1936) and of the priapial skeleton of the latter species by Aurich (1937) give detailed information to which I can add nothing except to mention that Bailey did not notice the vestigial fin rays which Aurich finds in the priapium of *G. mirabilis.* After very careful examination, I have identified both the loose post-anal group of rays and the group articulated with the posterior end of the axial bone in that species, thus supporting Aurich (see Aurich, 1937, Figs. 3 and 8). In the priapium of *Phenacostethus,* however, I have found no trace of fin rays. This fish is much smaller than *Gulaphallus* and the skeletal elements are correspondingly more delicate, which may in part account for the disappearance of such minute vestiges.

Pseudobranch

The pseudobranch, which Aurich says is lacking in phallostethids examined by him, including *Gulaphallus,* is present in both that species and in *Phenacostethus* in the form of two minute tufts (Myers, 1928) lying in a recess at the anterior dorsal end of the sub-opercular cavity. Microscopic study shows this organ to belong to type II of Granel's (1927) classification of teleost pseudobranchs, in that the epithelium of the branchial chamber surrounds each tuft separately, but does not extend between the secondary lamellae of the tuft. Each primary lamella, or tuft, is furnished with an afferent artery and is supported by a precartilage rod which is extremely delicate in *Phenacostethus.* The secondary lamellae are close together and contain the large acidophile cells typical of the pseudobranch.

Viscera

The general arrangement of organs in *Phenacostethus* is shown in Figs. 1 and 2. Comparison with Regan's (1916) figures of *Phallo-*



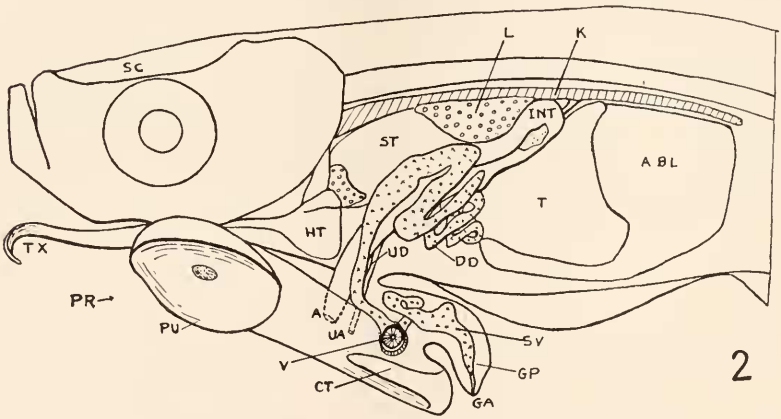


FIG. 1. *Phenacostethus smithi* ♂, internal organs in situ from the proctal side (× 16). Testis and portion of ductus deferens removed. The priapium is the large ventral appendage; several bones in it are outlined but Bailey's figures should be consulted for the complete skeleton.

FIG. 2. *Phenacosthethus* ♂, internal organs in situ from the aproctal side.

Abbreviations

A anus
ABL air bladder
AX axial bone
CT ctenactinium
DD ductus deferens
GA genital aperture
GG gas gland
GP genital papilla
HT heart
INT intestine
K kidney
L liver
PR priapium
PU sucker-like pulvinulus
S spleen
SC region of sensory canals
ST stomach
SV seminal vesicle
T testis
TX toxactinium
UA urinary aperture
UD urinary duct
V valve in ductus deferens

stethus dunckeri and with Villadolid and Manacop's (1934) figures of *Gulaphallus mirabilis* will give evidence of the great general similarity in the visceral anatomy of the group. Digestive and reproductive organs are located almost entirely in the space anterior to the air-bladder and, together with the excretory ducts, these systems terminate in apertures situated on the ventral surface of the throat region. It has already been pointed out (p. 59) that this extremely anterior position is associated with the male copulatory organ or priapium which is suspended from the head. The outlets are asymmetrically placed on the priapium; the anus and urinary duct open on one side, the proctal, while the ductus deferens ends in a genital papilla on the opposite or aproctal surface. As other workers have noted, this asymmetry is variable, that is, in some males the right side is proctal, in others, the left. In the female the apertures are mid-gular although no copulatory organ is present (Fig. 3).

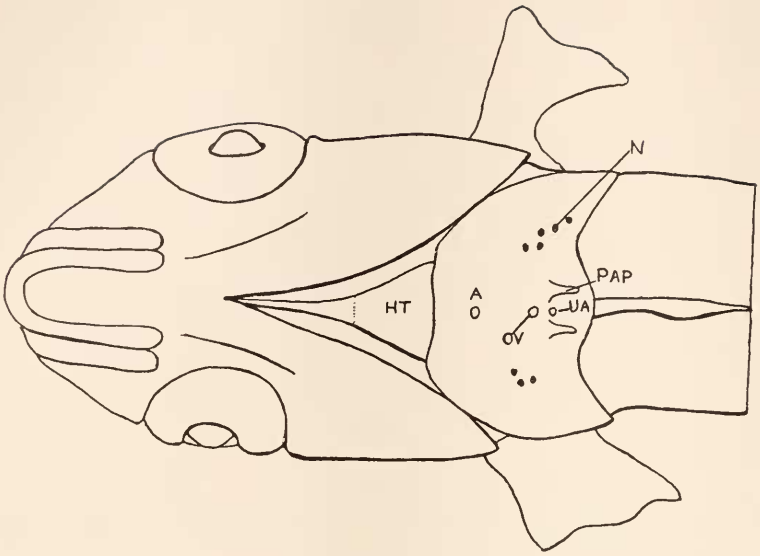
The following more detailed description applies to both *Phenacostethus smithi* and *Gulaphallus mirabilis* unless attention is called to one species only.

Endodermal Derivatives

The digestive tract is simple, short, and without pyloric caeca; a typical carnivorous type, as is borne out by Villadolid and Manacop in their analysis of the food of *Gulaphallus*. The intestine makes one complete coil in *Gulaphallus* and a partial coil in *Phenacostethus* (Fig. 1) before turning anteriorly toward the anus. The liver is large and lies close to the stomach and intestine (mainly on the aproctal side in the male); no gall bladder was identified. The pancreas is diffuse tissue, lying in the bend between stomach and intestine, with considerable fat scattered among its cells. Perhaps because of unsatisfactory fixation, no islet tissue is distinguishable. The spleen is partly embedded in the liver posterior to the stomach (Fig. 1). Thymus and thyroid glands are present. The large air bladder fills nearly one half of the body cavity. It lacks a pneumatic duct but is equipped in the anterior dorsal region with a vascular gas gland.

Nervous System

The size of the brain is large in proportion to the skull as in other minute fishes (TeWinkel, 1935). It was impossible because of poor fixation to determine whether the brain and cord are modified in relation to the priapium. Neuromasts are scattered on the surface of the body, particularly on the head region in *Gulaphallus;* a few are present in *Phenacostethus,* but here too, fixation was imperfect. Four large canals,



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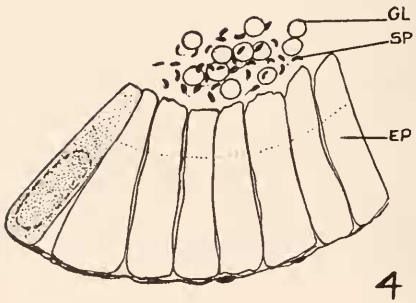

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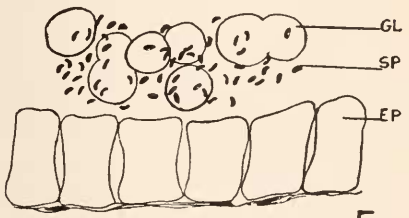

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FIG. 3. *Phenacostethus smithi* ♀, ventral view, anterior end (× 16). *A*, anus; *HT*, heart; *N*, neuromast; *O*, oviduct aperture; *PAP*, post anal papilla; *UA*, urinary aperture.

FIG. 4. Portion of proximal coil of ductus deferens in *P. smithi*. *Ep*, epithelium, showing in one cell probable position of nucleus; dotted line represents depth of lighter area in cells; *GL*, globules; *SP*, sperm head. Drawn with camera lucida under oil immersion lens.

FIG. 5. Portion of distal coil of ductus deferens from same section of *P. smithi* as Fig. 4. Epithelial cells are much lower and globules between sperm heads much larger than in the preceding figure. (Same magnification as above.)

with patches of sensory cells (which are probably lateral line organs) on their ventral inner walls, extend longitudinally on the snout and above the eyes in both species. These occupy considerable space between the skin and skull, and occasionally a homogeneous mass, possibly a cupula (Denny, 1937), is present in contact with the sense organ. The cavity containing the inner ear is proportionally large as in *Mistichthys*.

Kidney

The kidney in both species is a median organ which lies dorsal to the air bladder and digestive tract and diverges anteriorly into two sections which extend from below the occipital region of the skull up to the posterior level of the gills (Fig. 1). In *Phenacostethus*, and possibly in *Gulaphallus*, the urinary ducts are paired upon their exit from the kidney a short distance in front of the air bladder. They soon fuse, however, into a single duct which, in both species, passes between the intestine and gonad to open in the male on the priapium, directly behind the anus (Figs. 1, 2), and in the female, posterior to the oviduct in the gular region (Fig. 3).

Serial sections reveal one pair of pronephric glomeruli in both species. These lie dorsal to the heart and are directly attached to the dorsal aorta, which position is characteristic of the teleost pronephros. Poor fixation makes it impossible to tell whether the pronephric tubules are being replaced by lymphoid tissue, or whether they are functional, except the fact that the glomerular capillaries are full of blood cells, which suggests some function. The mesonephric glomeruli are smaller than the pronephric and are most numerous in that portion of the kidney anterior to the air bladder. The number of these glomeruli varies in *Phenacostethus* from 13 to 18, and in *Gulaphallus*, from 33 to 38, in the specimens where counts were made. In properly fixed material it would be more possible to determine, in so far as one could histologically, whether these fishes retain a functional pronephros in the adult, as is the case in *Fierasfer, Zoarces* (Emery, 1880), *Lepadogaster* (Guitel, 1906) and *Mistichthys* (TeWinkel, 1935). The number of glomeruli in *Phenacostethus* is only slightly greater than that in *Mistichthys* (which has 12 to 13 on the average) and it would be of interest to discover whether the ratio of glomerular volume to body surface is in any way comparable in these two unrelated minute teleosts.

Reproductive Organs

The ovary in the two species is single and lies along the anterior and ventral surface of the air bladder. In *Phenacostethus* there are on the

average fifteen maturing ova and the largest of these measure approximately 500 μ. Each ovum is surrounded by a thick porous zona radiata with surface filaments forming, but the method of filament attachment was not observed. The ovary of *Gulaphallus* contains about sixty maturing ova. The oviduct, in both species continuous with the ovary and doubtless formed by fusion of the mesovarium, is thick-walled and consists of a deep epithelial lining (possibly pseudo-stratified columnar) and a well-developed circular layer of smooth muscle. Its exterior opening lies just anterior to that of the urinary duct.

The breeding habits and development of *Gulaphallus* have been described by Villadolid and Manacop (1934). They demonstrate that the fish is oviparous but that fertilization is internal. No sperm are present in the oviducts of either species examined by me; the *Gulaphallus* material is immature and it is possible that *Phenacostethus* was collected at a time which was not the breeding season.

The testis is an unpaired organ, on the aproctal side partly wrapped around the anterior wall of the air bladder (Fig. 2). Seminiferous tubules open anterio-dorsally into a common collecting tubule. Because the structure of the ductus deferens differs considerably in the two species, each will be described separately.

In *Phenacostethus* all stages of spermatogenesis appear to be present in the testis. The ductus deferens is a long coiled tube, probably similar to that in *Phallostethus dunckeri* (Regan, 1913, 1916), forming a mass nearly equal to the testis in size. The tube is narrow in diameter for two or three coils, then increases to double its initial size, and finally extends into the priapium and into the genital papilla (Fig. 2). The epithelial lining of the tube is simple columnar, with tall cells in the narrower coils (Fig. 4), and cells about one-half this height in the large distal coils (Fig. 5), while that portion of the ductus deferens extending into the priapium is lined by low columnar epithelium. A valvular mechanism, greatly decreasing the lumen and lined by simple columnar epithelium, is present in the duct soon after it enters the genital papilla (Fig. 2). From this point the tube is slightly coiled, then dilates to form a seminal vesicle. Toward the tip of the papilla, the lumen is completely constricted and the wall seems to be composed of concentric layers of connective tissue, and very likely, some smooth muscle is also present.

The ductus deferens is crowded with spermatozoa in all specimens of *Phenacostethus* studied. In the narrow proximal coils of the tube (Fig. 4), there appear among the sperms small homogeneous globules which are, in all probability, secreted by the tall columnar epithelial cells of the lining. The size of these globules increases by one-half by

the time the large distal coils are reached (Fig. 5) and this increase may be the result of fusion of smaller globules. The staining capacity of these bodies varies: in Mallory's triple connective tissue stain, they are purplish in the proximal coils, but distally, brilliant red; in iron haematoxylin, brownish proximally, and distally gray or black; in alum haematoxylin and triosin, uniformly pink. These globules may be gelatinous spermatophores, but there seems to be no definite association between them and the sperms such as is described by Regan (1916) in two other phallostethids. Some spermatozoa may adhere to them, but there are quantities of free sperm in all parts of the duct. There is also a possibility that globules and sperm become united upon discharge, but study of living material is necessary to determine it.

The testis in the male specimen of *Gulaphallus* which was sectioned is large but immature.. The ductus deferens, within the body cavity a very narrow duct probably without secretory function, dilates in the posterior portion of the priapium to form a seminal vesicle. The duct is of course entirely devoid of sperm so that the presence of sperm packets, affirmed by Bailey who evidently studied more mature specimens of this species, was not observed. (See Pl. 3, fig. 3 in Villadolid and Manacop, 1934).

Study of the two phallostethids described in the preceding pages, in spite of the handicap of poorly fixed material, has shown that, histologically, there are no striking differences in the viscera that may be attributed to the priapium or to the displacement of the organ outlets to the throat region. The persistence of the pronephros, which may be functional in the adults, is of general interest because it occurs so rarely in teleosts; it would be of value to examine other members of the group to determine whether that organ is always present in phallostethids. The great difference in the structure of the ductus deferens in the two species is undoubtedly related to family specialization. Possibly, the adult male *Gulaphallus* develops a glandular groove externally (as in *N. lankesteri,* Regan, 1916) to compensate for lack of abundant secretion on the part of the sperm duct; such a groove was not seen, however, in the pre-adult male examined histologically.

Summary

The anatomy of the soft parts of two phallostethid fishes, *Phenacostethus smithi* and *Gulaphallus mirabilis,* has been studied.

1. Two groups of vestigial fin rays are present on the copulatory organs of *Gulaphallus* but are lacking on that of *Phenacostethus.*

2. A tuft-like pseudobranch, belonging to Granel's type II, is present in both species.

3. The viscera are located almost entirely anterior to the air bladder, and digestive, reproductive and excretory systems open externally on the sub-gular copulatory organ in the male and on the ventral surface of the gular region in the female. Liver, pancreas, spleen, thymus and thyroid are identified.

4. Neuromasts are scattered on the surface, and four canals, probably of the lateral line type, are present on the heads of both species.

5. The kidney contains one pair of pronephric glomeruli which may be functional. There are 13–18 mesonephric glomeruli in *Phenacostethus,* and 33–38 in *Gulaphallus.*

6. The ovary is single in both species and contains a relatively small number of ova. The oviduct, continuous with the ovary, is thick-walled and muscular.

7. The testis is a single mass. Seminiferous tubules open into a collecting tubule which continues as the ductus deferens. This duct forms a large coiled mass in *Phenacostethus* and is lined by simple columnar epithelium. Homogeneous globules, present among the sperms in the lumen, do not appear to form spermatophores. The ductus deferens in *Gulaphallus* is very narrow within the body cavity, but dilates to form a seminal vesicle in the copulatory organ.

LITERATURE CITED

AURICH, H., 1937. Die Phallostethiden. *Mitt. der Wallacea Expedition, Zweite Reihe, Intern. Rev. ges. Hydrobiol. u. Hydrograph.,* **34**: 263–286.

BAILEY, R. J., 1936. The osteology and relationships of the phallostethid fishes. *Jour. Morph.,* **59**: 453–483.

DENNY, M., 1937. The lateral-line system of the teleost, Fundulus heteroclitus. *Jour. Comp. Neur.,* **68**: 49–65.

EMERY, C., 1880. Fierasfer. *Atti della R. Accad. Lincei, Roma,* 3 ser., **7**: 167–254.

GRANEL, F., 1927. La pseudobranchie des poissons. *Arch. d'Anat. micr.,* **23**: 175–317.

GUITEL, F., 1906. Recherches sur l'anatomie des reins des quelques gobiésocidés. *Arch. Zool. Expér. Gén.* (Ser. 4), **5**: 505–700.

HERRE, A. W., 1925. Two strange new fishes from Luzon. *Philippine Jour. Sci.,* **27**: 507–513.

HERRE, A. W., 1926. Four new Philippine fishes. *Philippine Jour. Sci.,* **31**: 533–543.

MYERS, GEO. S., 1928. The systematic position of the phallostethid fishes, with diagnosis of a new genus from Siam. *Am. Mus. Novitates,* No. 295, *Am. Mus. Nat. Hist., New York.*

MYERS, GEO. S., 1935. A new phallostethid fish from Palawan. *Proc. Biol. Soc. Wash.,* **48**: 5–6.

MYERS, GEO. S., 1937. Notes on phallostethid fishes. *Proc. U. S. Nat. Museum,* **84**: 137–143.

REGAN, C. T., 1913. Phallostethus dunckeri, a remarkable new cyprinodont fish from Johore. *Ann. Mag. of Nat. Hist.* (Ser. 8), **12**: 548–555.

Regan, C. T., 1916. The morphology of the cyprinodont fishes of the sub-family Phallostethinae, with descriptions of a new genus and two new species. *Proc. Zool. Soc. London,* 1: 1–26.

Smith, H. M., 1927. The fish Neostethus in Siam. *Science, N. S.,* **65**: 353–355.

TeWinkel, L. E., 1935. A study of Mistichthys luzonensis with special reference to conditions correlated with reduced size. *Jour. Morph.,* **58**: 463–535.

Villadolid, D. V. and P. R. Manacop, 1934. The Philippine Phallostethidae, a description of a new species, and a report on the biology of Gulaphallus mirabilis, Herre. *Philippine Jour. Sci.,* **55**: 193–220.